1st. That in all persistent hyperemic conditions of the conjunctiva after cataract extraction with no other apparent symptom, the teeth may be the exciting cause that acting through the gyserian ganglion bring about the aforesaid result.

2nd. That as experience has taught me, the treatment of this condition is the extraction of the decayed teeth or imbedded roots.

I therefore recommend this practice in all protracted vaso-dilator disturbances after surgical interference in non-infected eyes, as a logical procedure worthy of being taken into consideration and backed with the experience of these few but well defined cases.

---

## NOTE ON THE SATURATION-POINT OF SERUM FOR NORMAL LIPOIDS AND CHOLESTERIN.

By CLARENCE QUINAN, M. D., San Francisco.

### MATERIALS.

*Serum.* A quantity of fresh blood was procured from one of the large packing-houses near San Francisco. Prime beef cattle were the source of supply. The blood was caught as it came from the throat incision in clean, dry glass jars. The stoppered, full receptacles at once were carried into the great refrigerator of the establishment, and after remaining there about 36 hours, the clear serum was aspirated into other containers. It was not considered necessary for the purposes of these experiments to collect the serum with full antiseptic precautions. The problem was rather to avoid contamination from unclean surfaces; in a word, to keep the specimens chemically clean. It seemed improbable that, in the brief experiment period, bacteria would multiply to a number sufficient to affect in a material degree the end results in tests of solubility, and, as a matter of fact, this element of error must have been very small indeed, for the control serum after 24 hours in the thermostat remained brilliant-clear, and to the naked eye presented nothing to indicate the presence of any considerable number of organisms. Bacteria were present, no doubt, but as yet probably had produced no extensive chemical changes.

*Normal Lipoids.* About one quart of fresh serum was mixed with five times its volume of absolute alcohol and the mixture was set aside for several days. The precipitated protein was then thrown upon a filter, and the yellowish, alcoholic filtrate was concentrated on the water-bath until of a sticky consistency. The relatively water-free residue was extracted with excess of absolute alcohol, the mixture was filtered free from inorganic salts, etc., again evaporated on the water-bath, and the residue then dried thoroughly at 100 Centigrade. The alcoholic extract of the serum obtained in the manner just described, was now thoroughly exhausted with absolute ether (distilled over metallic sodium), and the ether, finally, was distilled off, leaving behind a yellow, oily-looking mass of mixed lipoids. This substance was kept in a dessicator over solid calcium chloride until needed.

The fresh serum of healthy beef-cattle contains as a rule about 0.6% of fatty bodies which may conveniently be spoken of as lipoids. In a series of animals the amount varies but little. The mixed fats yield cholesterin upon saponification with alcoholic potash, and when the mass is oxidized with a mixture of sulphuric and nitric acids it gives a moderate reaction for phosphoric acid though, obviously, only a small amount of lecithin could thus be accounted for. The reaction of the mass is neutral.

*Cholesterin.* Pure cholesterin was prepared from human gallstones in the usual manner and the crystals were purified by recrystallization from absolute alcohol.

### METHOD.

The plan followed in this investigation was to add a weighed excess of normal lipoids or pure cholesterin to equal volumes of normal serum and serum previously modified in various ways by the addition of reagents, and then to determine, after a period of 24 hours in the thermostat, what portion of the lipoids, if any, had gone into solution. It was desired, especially, to alter the calcium relations of the serum, which, of course, could be effected without much chemical disturbance, and also to modify in a rather gross way the quality of the reaction, with a view to observe to what extent electrolytes play a part in the solution of the serum fats. The point upon which the greatest stress was placed, however, was that of the maximum solubility of normal lipoids and cholesterin in pure, unmodified serum. Accurate estimations of the ether-soluble elements, obviously, were indispensable. In work of this character the lipoid value of the normal serum remained, throughout, a constant, and was included in each extraction result; any increment of ether-soluble matter, therefore, could fairly be ascribed to the lipoid enrichment.

Two series of tests were prepared each of which consisted of six flasks. One of these served for the cholesterin tests, the other for those with normal lipoids. It will suffice to describe one such series in detail. The flasks were arranged as follows: 1. 25cc of normal serum, untreated, as control: 2. 25cc of normal serum. 3. 25cc of normal serum decalcified by the addition of .015 g. of ammonium oxalate in powder. (This is more than twice the theoretical amount of the ammonium salt required to precipitate the calcium, since, in a large number of gravimetric determinations of calcium as CaO in the ash of this serum the amount was practically constant at 0.0134 g. of CaO per 100 parts of serum). 4. 25cc of normal serum to which .030 g. calcium chloride was added. 5. 25cc of normal serum to which .5cc of n/2 hydrochloric acid was added, and, 6, 25cc of normal serum rendered hyperalkaline by the addition of 0.125 g. of sodium carbonate in substance. Each flask, depending upon the series, and with the exception of the control, received either 0.1 g. of cholesterin or an equal amount of normal lipoids. The sealed flasks remained for 24 hours in the thermostat. All were treated alike thereafter. The contents of all were passed at once through unglazed porcelain candles and

the resultant clear filtrates were used exclusively in all determinations of lipoids.

In the quantitative study of the fatty substances held in protein matter, protracted extractions in a Soxhlet or other similar apparatus are always necessary if one is to satisfy the most exacting analytical requirements, and the outlay of time unavoidably entailed where an extended series of such extractions must be carried out is of course very great. However, all but a small quantity of the lipoids are taken up by the alcohol or acetone employed in the preliminary precipitation, and for work in which fairly exact quantitative results are desired and in which extreme accuracy is not essential, Soxhlet extractions may be dispensed with and reliance placed upon the thorough use of absolute alcohol, acetone and ether by direct extraction. The latter method therefore was employed in the present study. Naturally, the conditions of experiment as far as possible were carefully controlled, and the extractions were done in duplicate.

Five cubic centimeter portions of the clear, Berkfeld filtrates were pipetted into flasks each of which contained 50 cubic centimeters of pure acetone and the flasks were allowed to stand for one week. The precipitated protein was then brought upon a filter, allowed to drain, rinsed with 20 cubic centimeters of acetone, and again allowed to drain. The protein mass was then thoroughly mixed with 25cc of absolute alcohol, and after this had passed through the filter, 20 cubic centimeters of ordinary pure ether were used to rinse down the filter and contents. This was the exact procedure in each instance. The solvents were distilled off on the water-bath, and the residue was dried to constant weight on it. The anhydrous residue was then exhausted with 75 cubic centimeters of absolute ether, this in turn was distilled off, and the lipoid residue finally obtained was dried to constant weight at 100° C. The values obtained are shown in tables I and II.

The unmistakable conclusion to be drawn from Table I is that cholesterin is nearly insoluble in normal serum. This interesting and important fact might have been safely predicted, *a priori,*— were it safe here to reason by analogy, from the chemical relations of cholesterin and its known insolubility in water. But then blood serum is by no means an ordinary aqueous solvent. There is oily matter dissolved in it, and oils are excellent solvents of many organic substances; ferments of one sort or another are present; esterification might be thought of and, perhaps, some allowance made for vitalism with its infinite possibilities. Had all the cholesterin been dissolved by the serum, in the five cubic centimeters of that fluid taken for the extraction twenty milligrams should have been recovered. The attempt to recover cholesterin from normal serum failed. The normal lipoid value remained unchanged. In one or two of the test mixtures, however, it will be seen that small gains over the normal were noted. But these tests represented modifications of reaction or mineral content such as would be unlikely to occur in fact, and it is very probable that the single high value noted in the hypoalkaline specimen falls within the limits of error inherent in the extraction method, and for that reason may be disregarded.

TABLE I.

Solubility of Pure Cholesterin in Serum. Duplicate analyses. 5cc of serum taken for each extraction. Figures show total extract soluble in absolute ether.

| | Total ether extract 5 cc (a) | Total ether extract 5 cc (b) | Mean | Cholesterin found |
|---|---|---|---|---|
| Normal serum (control) ..... | 0.0291 | 0.0274 | 0.0282 | |
| Normal serum and chol. ..... | 0.0290 | | 0.0290 | 0.0008 |
| Decalcif. serum and chol. ..... | 0.0300 | 0.0281 | 0.0290 | 0.0008 |
| Hypercal. serum and chol. ..... | 0.0228 | 0.0150 | 0.0189 | 0.0093 |
| $Na_2CO_3$ serum and chol. ..... | 0.0295 | 0.0302 | 0.0298 | 0.0016 |
| HCl. 5 cc n/2 serum and chol. .. | 0.0310 | 0.0332 | 0.0321 | 0.0039 |

TABLE II.

Solubility of Normal Lipoids in Serum. Duplicate analyses. 5cc of serum taken for each extraction. Figures show total extract soluble in absolute ether.

| | Total ether extract 5 cc (a) | Total ether extract 5 cc (b) | Mean | Normal lipoids found |
|---|---|---|---|---|
| Normal serum (control) ..... | 0.0291 | 0.0274 | 0.0282 | |
| Normal serum and lipoids..... | 0.0317 | 0.0334 | 0.0325 | 0.0043 |
| Decalcif. serum and lipoids..... | 0.0335 | 0.0329 | 0.0332 | 0.0050 |
| Hypercal. serum and lipoids..... | 0.0352 | 0.0301 | 0.0326 | 0.0044 |
| $Na_2CO_3$ serum and lipoids..... | 0.0358 | 0.0339 | 0.0348 | 0.0066 |
| HCl. 5 cc n/2 serum and lipoids | 0.0321 | 0.0326 | 0.0323 | 0.0041 |

Somewhat more positive results were obtained in the tests of normal lipoids, although, considering the origin of the experiment material, it would have been reasonable to anticipate larger values. Approximately twenty per cent. of the lipoids added to the serum as enrichment were found in solution. And the rate of gain, as may be seen in Table II, was pretty uniform throughout the series. But, since the lipoids used in the experiments were native constituents of the test serum, and hence were ideally adapted for the purposes of a saturation test, it follows that the value noted, *i. e.,* 0.0325 g., or 65%, represents, actually, the maximum solution number of a normal serum for its own proper lipoids. The mean value for this serum was 0.56%. Comparing values, therefore, it is evident that, in twenty-four hours the serum added almost exactly one-tenth of one per cent. to its lipoid content.

Conclusions:—

1. Pure cholesterin is very slightly if at all soluble in fresh bullock's serum.

2. Esterification of cholesterin does not occur to an appreciable extent in fresh bullock's serum *in vitro.*

3. The saturation-point of a serum for its own proper lipoids is only 0.1% in excess of the normal content.